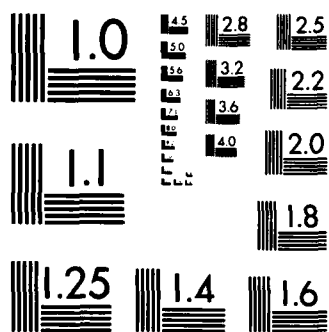END
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Genetic Characterization of
Insect Vectors of Diseases

Final Report

Jeffrey R. Powell

February 1984

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMENT
Fort Detrick, Frederick, Maryland 21701-5012

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We have analysed 120 population samples of Aedes aegypti for genetic variation at up to 22 protein-coding loci. Analysis of the data reveal distinct genetic-geographic groups.

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FINAL REPORT SUMMARY

We have analysed 120 population samples of Aedes aegypti for genetic
variation at up to 22 protein-coding loci. These samples originated from
75 localities throughout the world-wide distribution of the species. The
most important finding was that the species could be divided into 7 groups
based on geographic and subspecific criteria. These 7 groups are West Africa,
East Africa subspecies formosus, Asia, Southeast United States, Texas/Mexico,
South/CentralAmerica, Caribbean, and East Africa subspecies aegypti. These
groups are sufficiently distinct to allow assignment of a population of unknown
origin to one of the groups with a 90% or greater probability of correct assign-
ment.

Some of these samples have also been studied for their ability to transmit
yellow fever virus. Considerable variation for this trait was also found.

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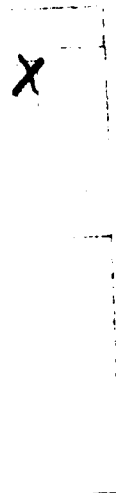
The main purpose of this contract was to analyse populations of Aedes

aegypti for genetic variability throughout its distribution. The species

is very widespread throughout the tropical and subtropical zones around

the world. Thus, there is a lot of territory to cover.

We have obtained about 120 samples from 75 different localities; from

some localities more than one sample were obtained. These samples represent

as well as possible the world-wide distribution of the species. Generally

a sample consisted of 50 to 300 individuals.

Genetic variation was determined at up to 22 enzyme-coding loci by

the method of gel electrophoresis. Individuals collected in nature were

either electrophoresed directly or F1 offspring were used in instances where

too few individuals from nature were obtained. Thus we avoided, as much as

possible, artifacts inherent in long-term laboratory rearing. There were

about 50 alleles at the 22 loci studied. The data consist of allele frequencies

at each locus for each sample. Thus the total data set is about 6,000 allele

frequencies.

In order to make this large amount of data comprehensible, we have used

various analytical procedures, some of which are fairly standard for population

genetic analysis, while others were developed by us to specifically answer

questions pertinent to Aedes aegypti.

While not directly supported by this contract, some samples obtained under

it were used in another study of the genetic basis of yellow fever transmission.

The attached preprint of a paper from a symposium summarizes this work.

All the work supported by this contract has been published. Reprints have

been supplied in previous reports. Appended here is a complete list of publi-

cations which eminated from work supported by this contract.

1980   Powell, J.R., W. Tabachnick and J. Arnold. 1980. Genetics and the origin
        of a vector populations: _Aedes aegypti_, a case study. _Science_
        208:1385-1387.

1982   Powell, J.R., W. Tabachnick and G.P. Wallis. 1982. _Aedes aegypti_ as a
        model of the usefulness of population genetics of vectors. In:
        Recent Developments in the Genetics of Insect Disease Vectors, a
        Symposium Proceedings, pp. 396-412, edited by W.W.M. Steiner _et al._,
        Stipes Publishing Co., Champaign, IL.

        Tabachnick, W., T.H.G. Aitken, B.J. Beaty, B.R. Miller, J.R. Powell and
        G.P. Wallis. 1982. Genetic approaches to the study of vector
        competency in _Aedes aegypti_. In: _Recent Developments in the Genetics_
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        Wallis, G.P. and W.J. Tabachnick. 1982. Linkage of isocitrate
        dehydrogenase with sex and two lethals in _Aedes aegypti_. _Journal of_
        Heredity 73:291-294.

1983   Wallis, G.P., W.J. Tabachnick, and J.R. Powell. Macrogeographic genetic
        variation in a human commensal: _Aedes aegypti_, the yellow fever
        mosquito. _Genetical Research_ 41:241-258.

1984   Wallis, G.P., W.J. Tabachnick, and J.R. Powell. Genetic heterogeneity
        among Caribbean populations of _Aedes aegypti_. _Journal of Tropical_
        Medicine and Hygeine 33:492-498.

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        Powell, J.R. Geographic genetic differentiation and arbovirus competency:
        Aedes aegypti and yellow fever. Proceedings of a symposium, _Genetic_
        Approaches to the Study of Parasites and Disease Vectors edited by M.
        Coluzzi, Rome, December 1983.

Geographic Genetic Differentiation and Arbovirus Competency:

Aedes aegypti and Yellow Fever

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In diploid, sexually reproducing organisms, no two individuals are genetically identical (with the possible exception of monozygotic twins). Thus, while it is possible to define a species as a coherent unit, one must always remember such units are composed of populations of diverse individuals. In parasitology when one designates a species as the pathogen, vector, or host it must be borne in mind that considerable variation among individuals exists in each. Some of this variation may have considerable relevance with respect to disease transmission. One need only mention drug resistence in *Plasmodium* and sickle-cell anemia in humans to underscore this point.

Several years ago we embarked on a population genetic analysis of *Aedes aegypti*, the major vector of urban yellow fever. Our initial interest in the species was due primarily to its interesting evolutionary history. It has long been recognized as a polytypic species, i.e. has genetic variation for morphological traits. Further studies revealed genetic variation for a number of physiological and behavioral traits as well. Ecological studies, especially in East Africa, identified what is the probable ancestral sylvan form which gave rise to the domestic form which in turn spread around the world as a human commensal. Because the most widespread form is associated almost exclusively with humans, educated guesses can be made as to routes and times of introduction and levels of gene flow among populations. These factors, plus the fact it is quite easy to rear in the laboratory, made *Aedes aegypti* an attractive species for population genetic studies.

Coincidently, there is a research group at Yale University interested in genetic variation in insect vector ability to transmit arboviruses. These researchers are members of the Yale Arbovirus Research Unit (YARU) in the Department of Epidemiology and Public Health. For the past four years we have been involved in cooperative research with this group in an attempt to combine knowledge of the population genetic structure of *Aedes aegypti* with studies of yellow fever virus vectorial capacity. In

this contribution, I summarize the results we have obtained.

At this point, I must emphasize that the work reported here is the result of the combined efforts of several workers. Members of the YARU team include T.H.G. Aitken, B. J. Beaty, L. Lorenz, B. R. Miller, and R. Tesh. In my laboratory in the Yale Department of Biology the main contributors have been W. J. Tabachnick, G. P. Wallis, and G. D. Amato.

POPULATION GENETICS OF Aedes aegypti

As mentioned above, genetic variation in A. aegypti had been known for some time. The type of variation studied had been morphological, physiological, and behavioral. While these data indicated genetic differentiation among populations, it is difficult to use such data to quantify genetic differentiation. Therefore, we chose to use isozyme techniques to study patterns of genetic variation and differentiation in this species. Data resulting from such studies have two especially useful properties: First, the data are quantifiable; one actually counts gene frequencies and obtains exact numbers for the sample. Second, several genes distributed all over the genome can be assayed. This latter fact means that the results obtained from such studies probably reflect the overall genetic differentiation of populations. The various markers (genes) reflect the differentiation at that locus and segments of the chromosome surrounding it. The genes themselves, coding for enzymes, may not be important per se; rather they are convenient markers which hopefully reflect the genome as a whole. If so, then the patterns of variation discerned for isozymes may be reflected in variation in other traits, such as efficiency of disease transmission. As will become clear, this is at least partly true for A. aegypti and yellow fever.

We initially studied about 25 enzyme-coding loci in A. aegypti. Of these 10 or 11 proved to be polymorphic and therefore useful in determining patterns of differentiation. To date, we have analyzed more than 100 samples from about 70 localities throughout the distribution of the species; more than one sample was obtained from several localities. Each of the 100 samples averages about 50 individuals (100 genes) analyzed for variants at each locus. Thus one can appreciate that the data from these studies is rather extensive; presenting table after table of gene frequencies would not be particularly enlightening. Rather we have attempted to use several statistical methodologies to summarize the data for various purposes.

One will be presented here.

In analyzing genetic variation in vectors one useful question which may be asked is whether there is sufficient differentiation to be able to assign a population of unknown origin to the region from which it originated. That is, do populations occupying different regions of a species distribution have gene frequency differences of great enough magnitude to discriminate among regions? There exists a statistical procedure designed to answer such questions; this is the stepwise multivariate discriminant analysis. In order to use this procedure one must first define the groups to be discriminated. Using other analytical procedures, we defined eight genetically distinct groupings of A. aegypti populations. These are: East Africa subspecies aegypti (abbreviated EAA), East Africa subspecies formosus (EAF), West Africa (WA), Asia, Southeast United States (SEUS), Texas and Mexico (TEXMEX), Central and South America including Trinidad (CSA), and the Caribbean (CAR).

Once groupings are defined, the discriminant analysis determines the composite variable based on allele frequencies which best discriminates the groups. The variable is given coefficients which maximizes the ratio (F) of between group variance to within group variance. The resulting variable is called the first canonical variable. The variance in gene frequencies correlated to this first canonical variable is removed from the data and a second composite variable is determined based on the remaining variance. The procedure can proceed until all the variance in gene frequencies is exhausted. Fortunately, for our data the first two canonical variables account for a large fraction, up to 90%, of the total variance. This means that the essence of the analysis can be displayed in a two dimensional graph.

Figure 1 is an illustration of such an analysis on the more than 60 localities we have studied. (If more than one sample has been analyzed for a locality, mean gene frequencies across samples were used.) As can be seen, EAA localities are widely separated from all the others, which are not well separated toward the left of the

graph. Because of the distortion generated by the inclusion of EAA, one can rerun the analysis omitting the three EAA localities. The results are in Figure 2. Six of the remaining seven groups initially defined are separated. TEXMEX and CSA are indistinguishable. Therefore we conclude that it may not be valid to consider these separate groups. Based on this analysis then, we can legitimately define seven genetically distinct groups of populations of *A. aegypti*. In most cases there is a greater than 90% probability of assigning a population of unknown origin to the correct group based on allele frequencies at these ten loci. (For more details of the data and procedures, see Wallis et al., 1983, and references therein.)

We should note that in defining genetically distinct groups in this manner, groupings may change as more samples are obtained. Groups may be merged and others erected. However, with our *A. aegypti* data, the groupings have remained remarkably stable as we have added more samples. We first attempted to define groups and subject them to discriminant analysis when we had 34 samples from 28 localities (Powell et al., 1980). At that time we defind seven groups, the same as before except for TEXMEX for which we had no samples. When samples from Texas and Mexico were obtained, we noted that allele frequencies were distinct from other U.S. populations and therefore erected a new group. Now with more South American samples, it appears that perhaps this TEXMEX should be merged with CSA. Except for this change, nearly tripling the number of samples has not disrupted the groupings. This increases our confidence that the groups are real and not artifacts of the analytical procedure.

YELLOW FEVER COMPETENCY

Twenty-four of the population samples have also been studied for their competency to be infected with yellow fever virus (Tabachnick, et al., in preparation). Mosquitoes were fed on a suspension of virus, and allowed to incubate for 14 days at $26°$ C. Heads were then squashed and the presences of virus detected by fluorescent antibodies. (Details will be presented elsewhere). Table 1 shows the results. The rate of infection varied from 7 to 57%. Table 2 is a statistical analysis of these data. In this Table we have included populations which had been in laboratory culture for three generations or less. The reason is that we have evidence that under culture, strains change genetically.

While the correlations are far from perfect, the genetic groupings we defined and the rates of infectivity are related. The groups CAR, CSA, EAF, tend to be high in infectivity while WAF and TEXMEX are low. ASIA falls intermediate. SEUS is quite heterogeneous, having populations both relatively high and relatively low.

This nonrandom listing of populations in Table 2 argues for a genetic component in determining infectivity. We would not argue that the isozymes themselves are involved in infectivity. However they seem to be marking areas of the genome which are important.

I should also mention that we have done selection experiments trying to derive populations higher and lower in susceptibility to yellow fever infection. These attempts have been partially successful. However, such studies, like those presented in Tables 2 and 3, do give indications of genetically determined variation for this trait which is probably quite complex. That is, many genes each with small effects are likely to be involved. This makes more sophisticated genetic analysis very difficult.

It is interesting to note that of all the genetic studies on arbovirus competency in mosquitoes (reviewed in Gubler, et al., 1982) none have found simple genetic

control. This is in contrast to studies on other parasitic diseases such as malaria and filariasis for which single mendelian factors have been implicated in determining vectorial competency.

ACKNOWLEDGMENTS

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TABLE 1

Rates of infectivity with yellow fever virus for populations of _Aedes aegypti_.

GROUP	POPULATION	GENERATION TESTED	NUMBER INFECTED ——————— NUMBER TESTED	% INFECTED
WAF	Dakar, Senegal	F2	36/334	11
	N'goye, Senegal	F3	2/30	7
	Gambia	F6	3/11	27
EAF	Kampala, Uganda	F14	5/59	8
	Kombeni, Kenya	F8	36/107	34
EAA	Kwa Dzivo, Kenya	F3	104/184	57
	Majengo, Kenya	F3	65/222	29
ASIA	Amphur, Thailand	F29-31	97/350	28
	Bangalore, India	F3	19/84	23
	Fiji	F1	19/49	22
TEXMEX	Austin, Texas	F3	35/119	29
	Galveston, Texas	F1	3/19	16
	Houston, Texas	F1	7/34	21
	Weslaco, Texas	F1	2/13	15
	Victoria, Mexico	F3-4	39/198	20
SEUS	Abbeville, Louisiana	F1	4/33	12
	Beaumont, Texas	F1	6/23	26
	Vero Beach, Florida	F0	14/34	41
CSA	Escuintla, Guatemala	F1	82/258	32
	Malaga, Colombia	F0	11/24	46
	Santa Cruz, Bolivia	F4	31/99	31
	Trinidad	F1,4	72/173	42
CAR	Montserrat	F0	16/30	53
	Puerto Rico	F3	17/50	34

TABLE 2

Populations in Table 2 in decreasing order of susceptibility to oral-infection with yellow fever virus. Vertical lines connect populations showing no significant (p 0.05) difference in infectivity by a chi-squared test.

| POPULATION	GROUP
Kwa Dzivo	EAA
Montserrat	CAR
Malaga	CSA
Vero Beach	SEUS
Trinidad	CSA
Puerto Rico	CAR
Santa Cruz	CSA
Escuintla	CSA
Majengo	EAA
Austin	TEXMEX
Beaumont	TEXMEX
Bangalore	ASIA
Fiji	ASIA
Houston	TEXMEX
Victoria	TEXMEX
Galveston	TEXMEX
Weslaco	TEXMEX
Abbeville	SEUS
Dakar	WAF
Ngoye	WAF

FIGURE 1

Canonical plot of _Aedes aegypti_ populations based on 10 polymorphic isozyme loci. Each population is represented by the initial letter of its group, except x for EEA; overlap of populations is represented by *. Numbers are the mean position for the group.

FIGURE 2

Same as Figure 1 omitting EEA populations.

Fig 2

Canonical variable 1

Canonical variable 2

CAR

EAF

WA

SEUS

ASIA

TEXMEX
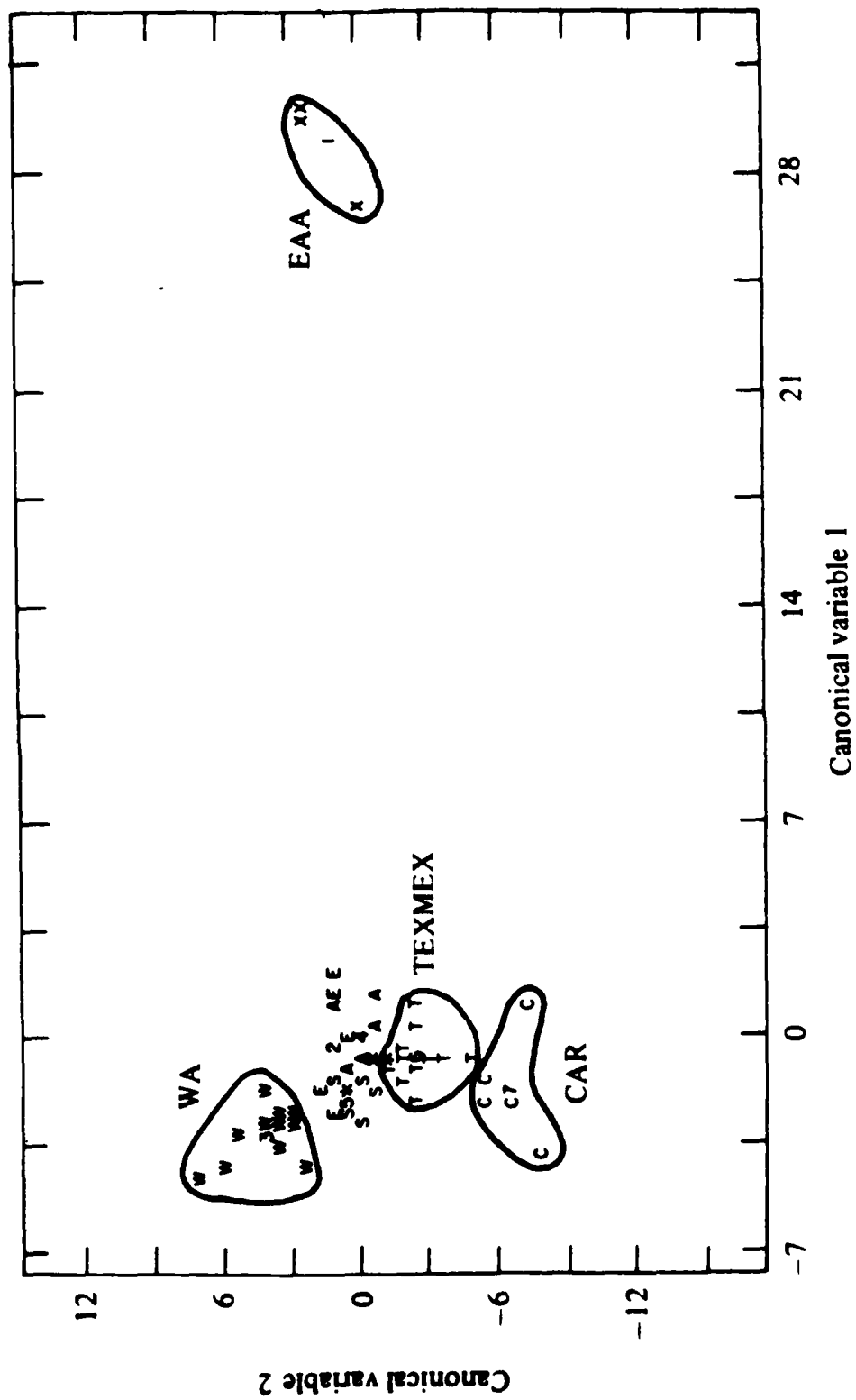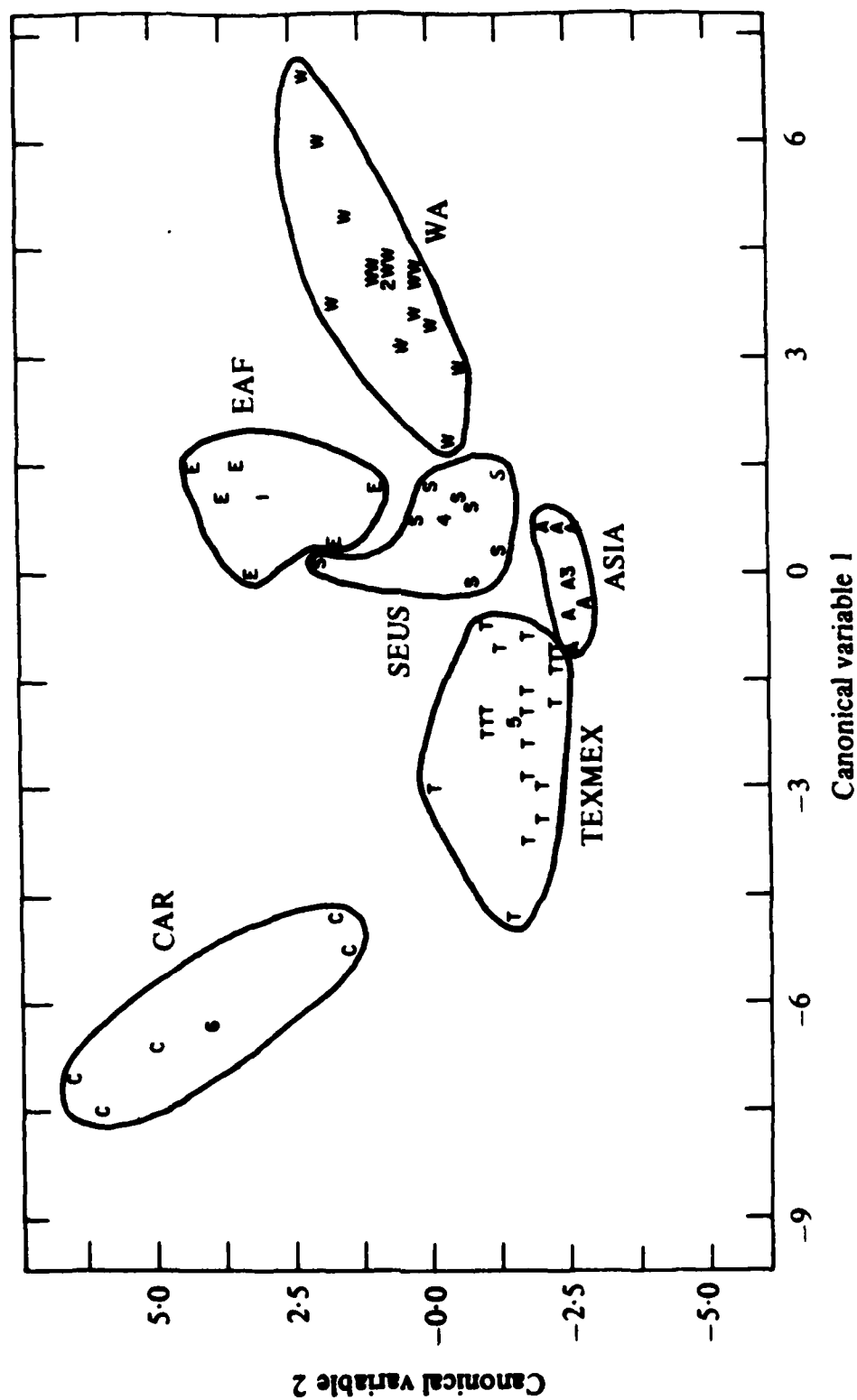
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